



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2018

---

## **Encephalitozoon cuniculi infection in Barbary striped grass mice (*Lemniscomys barbarus*)**

Kitz, Sarah ; Grimm, F ; Wenger, Sandra ; Hatt, Jean-Michel ; Kipar, Anja ; Hetzel, Udo

**Abstract:** *Encephalitozoon cuniculi* is an obligate intracellular microsporidian parasite that commonly induces subclinical infections in rabbits, but occurs also in a range of other species, including various rodents, carnivores, humans and birds. The present report describes encephalitozoonosis in a group of captive Barbary striped grass mice (*Lemniscomys barbarus*) in a zoo collection. The aetiology was confirmed by immunohistochemistry and PCR with subsequent sequencing. The source of infection is not known.

DOI: <https://doi.org/10.17236/sat00166>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-151990>

Journal Article

Published Version

Originally published at:

Kitz, Sarah; Grimm, F; Wenger, Sandra; Hatt, Jean-Michel; Kipar, Anja; Hetzel, Udo (2018). *Encephalitozoon cuniculi* infection in Barbary striped grass mice (*Lemniscomys barbarus*). *Schweizer Archiv für Tierheilkunde*, 160(6):394-400.

DOI: <https://doi.org/10.17236/sat00166>

# *Encephalitozoon cuniculi* infection in Barbary striped grass mice (*Lemniscomys barbarus*)

S. Kitz<sup>1</sup>, F. Grimm<sup>2</sup>, S. Wenger<sup>3,\*</sup>, J.M. Hatt<sup>3</sup>, A. Kipar<sup>1</sup>, U. Hetzel<sup>1</sup>

<sup>1</sup>Institute of Veterinary Pathology, <sup>2</sup>Institute of Parasitology, and <sup>3</sup>Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Switzerland

<https://doi.org/10.17236/sat00166>

Received: 08.02.2018  
Accepted: 01.05.2018

## Summary

*Encephalitozoon cuniculi* is an obligate intracellular microsporidian parasite that commonly induces subclinical infections in rabbits, but occurs also in a range of other species, including various rodents, carnivores, humans and birds. The present report describes encephalitozoonosis in a group of captive Barbary striped grass mice (*Lemniscomys barbarus*) in a zoo collection. The aetiology was confirmed by immunohistochemistry and PCR with subsequent sequencing. The source of infection is not known.

**Keywords:** Encephalitozoonosis, microsporidia, Barbary striped grass mice (*Lemniscomys barbarus*)

## Zusammenfassung

*Encephalitozoon cuniculi* ist ein obligat intrazelluläres Mikrosporidium, das häufig subklinische Infektionen bei Kaninchen hervorruft. Es kann jedoch auch eine Reihe anderer Spezies, wie zum Beispiel Nagerartige, Karnivoren, Menschen und Vögel infizieren. Der vorliegende Bericht beschreibt eine Encephalitozoonose bei Berber-Streifengrasmäusen (*Lemniscomys barbarus*) in einem zoologischen Garten. Der Erreger wurde mittels Immunhistochemie und PCR sowie anschließender Sequenzierung nachgewiesen. Die Infektionsquelle ist nicht bekannt.

**Schlüsselwörter:** Encephalitozoonose, Mikrosporidien, Berber-Streifengrasmäuse (*Lemniscomys barbarus*)

## Introduction

*Encephalitozoon cuniculi* (*E. cuniculi*) is a zoonotic, unicellular, spore forming parasite with an obligate intracellular development, belonging to the phylum Microsporidia, class Microsporea and order Microsporidia<sup>1–3</sup>. Microsporidia were originally thought to be protozoa but are now believed to be a sister group of fungi. The host range of *E. cuniculi* is wide<sup>4</sup>, but wild and domestic rabbits (*Oryctolagus cuniculus*) are most commonly and predominantly subclinically infected<sup>1,2</sup>. So far, four genotypes have been identified based on the number of 5'-GTTT-3' repeats in the ribosomal internal transcribed spacer (ITS) region of the rDNA gene; a "rabbit strain" (genotype I) identified in rabbits, wild birds, non-human primates and humans; a "mouse strain" (genotype II) and a "dog strain" (genotype III), both with broad host ranges that include rodents, birds, carnivores, primates, humans (genotype III), and a "human strain" (genotype IV) described in humans, cats and dogs<sup>5–7,4</sup>.

Clinical disease is mainly seen in immunosuppressed individuals, but has also been reported in immunocom-

petent hosts including rodents, carnivores, humans and birds; it is usually associated with neurological signs and inflammatory lesions in brain and kidneys<sup>8,9</sup>. Infected animals intermittently shed infective spores with urine, sputum and/or faeces. Hosts are mainly infected via the oral route, i.e. by ingestion of water or food contaminated with microsporidian spores, via inhalation, or transplacentally<sup>9–11</sup>. In the host, microsporidia develop by several cycles of merogony, followed by sporogony in the cytoplasm of infected cells. Previous studies have shown that in the early stage of infection, *E. cuniculi* infects macrophages as these phagocytose the parasites<sup>12,13</sup>. Macrophages are also responsible for their systemic spread, which occurs particularly in immunosuppressed hosts<sup>14</sup>. In addition, *E. cuniculi* infects a wide range of cells, such as vascular endothelial cells particularly in the brain, and tubular epithelial cells in the kidney<sup>15</sup>, leading to shedding via the urine<sup>16</sup>. Infectious spores use a coiled polar tube to enter host cells; this is ejected during invasion and penetrates the host cell, directly inserting the sporoplasm into the cytoplasm<sup>17–19</sup>.

\* Author's present address: Kleintierpraxis Laupeneck, Laupenstrasse 33, Bern, Switzerland

## Case Report

A 2-week-old male Barbary striped grass mouse (*Lemniscomys barbarus*) was found dead in its enclosure in a zoological garden. The animal had not shown any clinical signs prior to death. The gross post mortem examination did not reveal any relevant macroscopic changes.

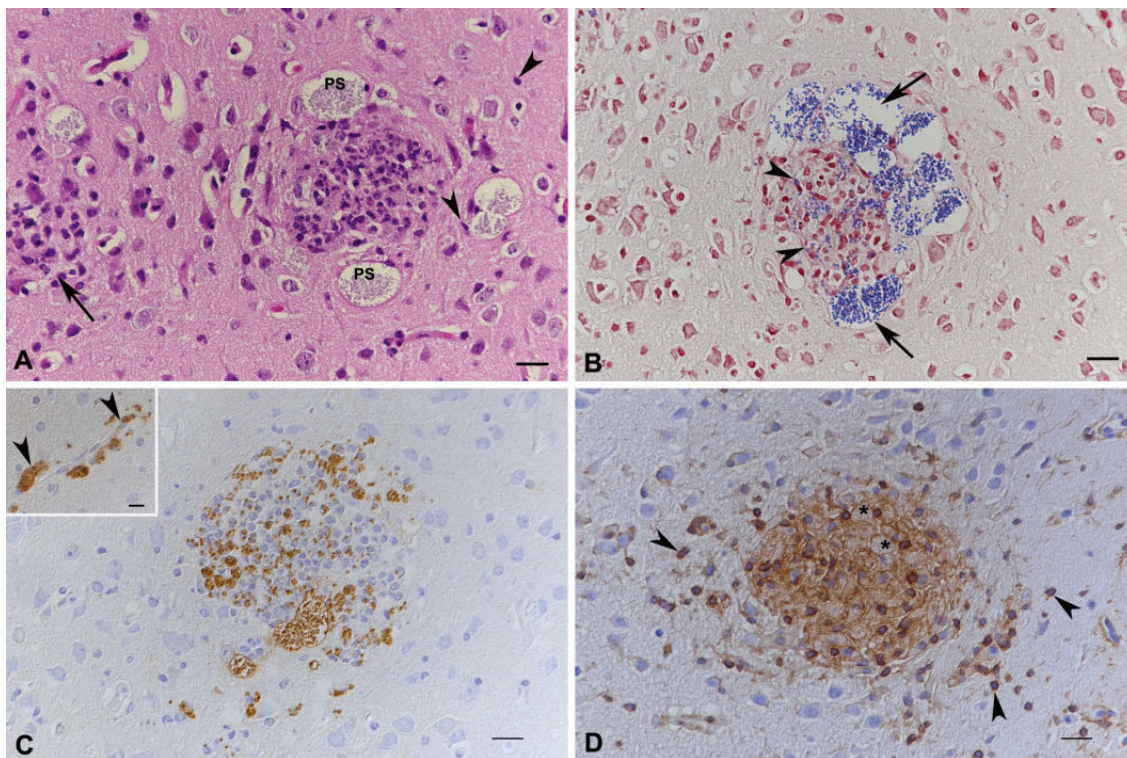
The histologic examination revealed multifocal inflammatory processes in brain, lung, spleen, liver, kidneys and pancreas, with a variable number of pseudocystic structures of up to 120 µm in diameter, or accumulations of individual, approximately 2 mm, acid fast, Gram-, and PAS-positive protozoan-like structures both within cells and extracellular (Fig. 1A, B). In the brain these structures were found within multifocal granulomatous infiltrates in cortex and brain stem. The infiltrates were generally located in close proximity to blood vessels and associated with focal microglial cell activation and accumulations (microglial nodules) (Fig. 1C, D). In addition, protozoan-like structures were also detected within the cytoplasm of numerous intact vas-

cular endothelial cells (Fig. 1C, inset). In the cerebral cortex, the granulomatous inflammation was accompanied by diffuse perivascular parenchymal and meningeal lymphoplasmacellular infiltration. The spleen, lungs and kidneys also exhibited a (multi)focal granulomatous inflammation which contained these structures within macrophages (Fig. 2A). In the lungs, similar structures were also observed in endothelial cells, pneumocytes, bronchial epithelial cells and alveolar macrophages. The liver, heart, pancreas and kidneys displayed mild to moderate multifocal lymphoplasmacytic infiltrates with intracellular protozoan-like structures within macrophages, endothelial cells, and parenchymal cells, i.e. hepatocytes and renal tubular cells (Fig. 2B, C). Based on the type of inflammatory lesions and the morphology of these structures, *E. cuniculi* or *Toxoplasma gondii* infection was suspected.

The examined animal originated from a group of approximately 20 Barbary striped grass mice that were housed in a savanna to grassland-like vivarium exhibit. Their diet consisted mainly of a mixture of seeds, vege-

*Encephalitozoon cuniculi* infection in Barbary striped grass mice (*Lemniscomys barbarus*)

S. Kitz et al.



**Figure 1:** Animal 1. Brain, cortex; granulomatous infiltrates with intralesional protozoan-like structures. **A.** Pseudocystic structures (PS) are present within cells, and there is evidence of microglial nodule formation (arrow) and microglial activation (arrowheads). HE stain. **B.** The protozoan-like structures are Gram-positive and are found arranged in the large pseudocystic structures (arrows) or, individually and as smaller groups, within macrophages (arrowheads) in the granulomatous infiltrates. Gram stain. **C.** The protozoan-like structures react strongly with a polyclonal rabbit anti-*E. cuniculi* antibody. Inset: Protozoan-like structures are also seen within vascular endothelial cells (arrowheads). **D.** The granulomatous infiltrate is mainly comprised of Iba-1+ macrophages/microglial cells (\*: pseudocystic structures). The adjacent parenchyma exhibits activated, Iba-1+ microglial cells (arrowheads). **C, D.** Polyclonal rabbit anti-*E. cuniculi* antibody (C) and polyclonal rabbit anti-Iba-1 antibody (D), horseradish peroxidase method (Envision; Dako); antibody binding visualised with diaminobenzidine, haematoxylin counterstain. **A-D.** Bars = 10 µm.



*Encephalitozoon cuniculi*  
infection in Barbary  
striped grass mice  
(*Lemniscomys barbarus*)

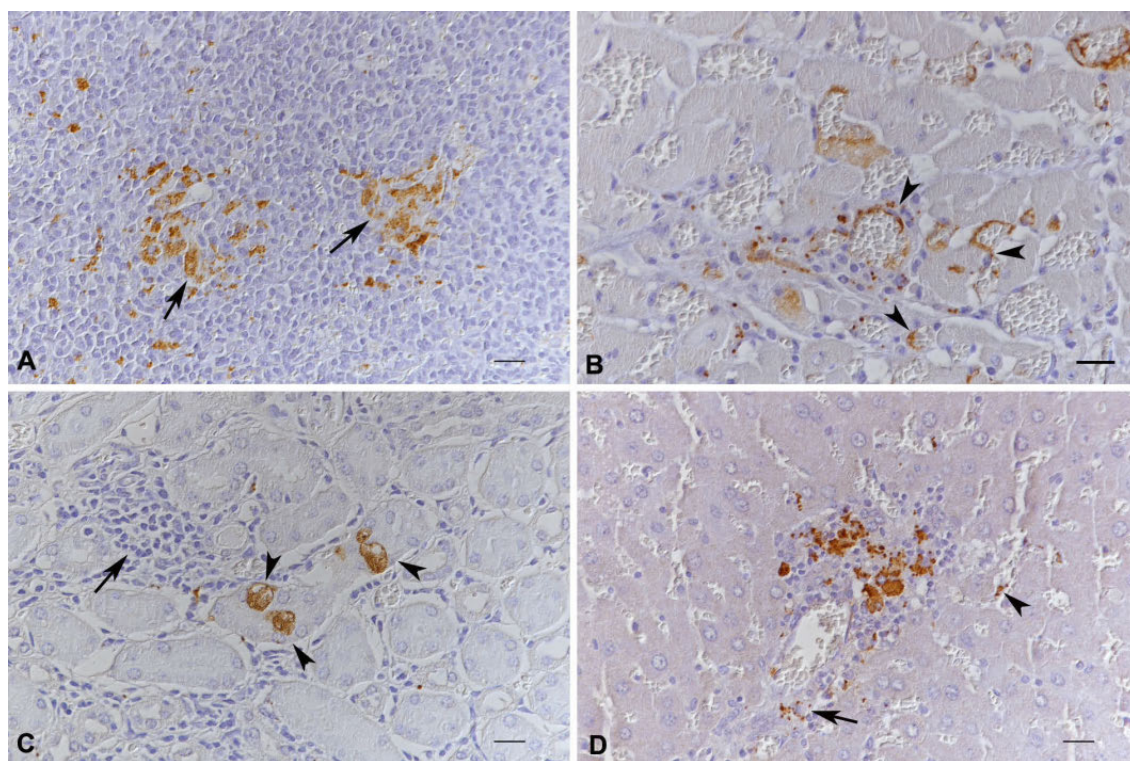
S. Kitz et al.

table and fruits, to a lesser extent also of living insects. The mice were not in direct contact with other animals. In order to assess the relevance of the infection for the colony, two further, healthy mice, a male (animal 2) and a female (animal 3) of approximately 13 weeks, were electively euthanised and examined; samples were taken for histology and transmission electron microscopy (TEM). The histological examination of both animals revealed multifocal, predominantly mononuclear inflammatory processes. In animal 2, these were restricted to multifocal periportal lymphoplasmacytic infiltrates in the liver, with rare intralesional protozoan-like structures within Kupffer cells and hepatocytes (Fig. 2D). Animal 3 exhibited similar changes in the liver and focal lymphoplasmacellular infiltrates in the renal pelvis, with a few similar structures in intact tubular epithelial cells, partially associated with inflammatory infiltration. However, similar to animal 1, it also showed a marked granulomatous encephalitis with numerous protozoan-like structures. Again, cortex and brain stem were affected most intensely. The ultrastructural examination of the cortex revealed intralesional protozoan-like spores with an electron dense spore wall (exospore), chitinous radiolucent endospores, and a characteristic coiled polar

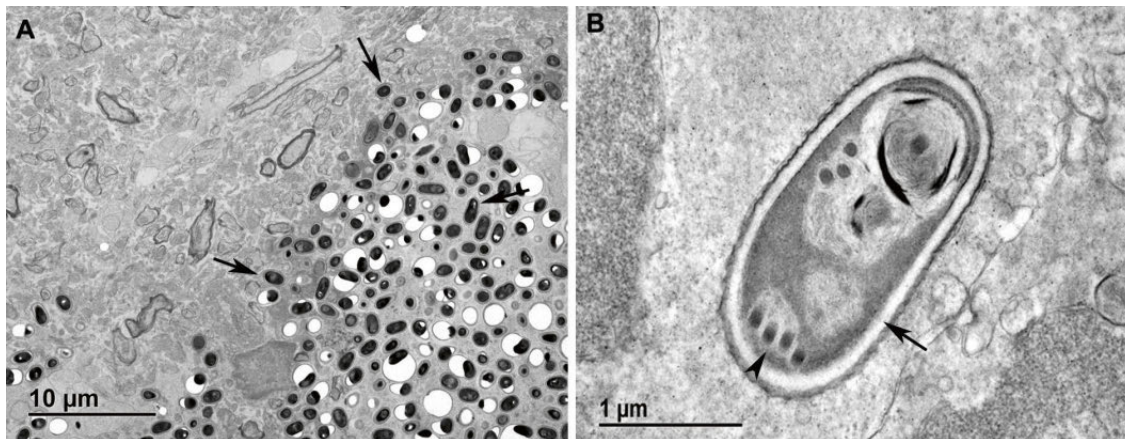
filament (polar tube) arranged in rows with coils (Fig. 3), features consistent with *E. cuniculi*<sup>20</sup>.

Immunohistochemistry (IHC) was performed on paraffin sections of lesions in all three animals, using protocols routinely applied for the detection of *T. gondii*, *Neospora caninum* or *E. cuniculi* and to stain macrophages and microglial cells (Iba-1+), and appropriate positive and negative controls. Briefly, a polyclonal rabbit anti-*T. gondii* (Neomarkers, Fremont, USA), a non-commercial mouse monoclonal anti-*N. caninum*, a non-commercial polyclonal rabbit anti-*E. cuniculi* and a polyclonal rabbit anti-Iba-1 (Wako Chemicals GmbH, Neuss, Germany) antibody were used, following previously published protocols<sup>21–24</sup>.

The intralesional protozoan-like structures were strongly labelled with the antibody against *E. cuniculi* in all three animals (Fig. 1C, 2); they also reacted with the anti-*T. gondii* antibody, which is not specific for *T. gondii*, but known to also react with other apicomplexans, including *N. caninum*, *Isospora* spp. or *Eimeria* spp.<sup>20</sup>. Staining for *N. caninum* yielded no reaction.



**Figure 2:** A–C. Animal 1. A. Spleen. Granulomatous splenitis with abundant protozoan-like structures within intralesional macrophages (arrows). B. Heart. Protozoan-like structures are found within numerous endothelial cells of small vessels (arrowheads). C. Kidney with several pseudocystic structures within intact tubular epithelial cells (arrowheads). The adjacent interstitium exhibits a focal lymphoplasmacellular infiltration (arrow). D. Animal 2. Liver. Protozoan-like structures are found within mononuclear infiltrates in portal areas and within scattered hepatocytes (arrow) and Kupffer cells (arrowhead). A–D. Polyclonal rabbit anti-*E. cuniculi* antibody, horseradish peroxidase method (Envision; Dako); antibody binding visualised with diaminobenzidine, haematoxylin counterstain. A–D. Bars = 10 µm.



**Figure 3:** Animal 3, brain, cortex. Transmission electron micrograph. **A.** Intralosomal protozoan-like spores (arrows). Bar = 10 µm. **B.** Individual spore, 1.5 × 2.0 µm in size, with a protective exo- and endospore (arrow), and a characteristic coiled polar filament arranged in a row with 4 coils (arrowhead). Bar = 1 µm.

*Encephalitozoon cuniculi*  
infection in Barbary  
striped grass mice  
(*Lemniscomys barbarus*)

S. Kitz et al.

Polymerase chain reactions (PCR) specific for *T. gondii* (RE sequence)<sup>25</sup>, *N. caninum* (Nc5 region)<sup>21</sup> and *E. cuniculi* (small-subunit rRNA gene)<sup>26</sup> were performed on fresh tissue samples of liver, kidney and brains of all three animals. These were negative for *T. gondii* and *N. caninum*, but positive for *E. cuniculi*. Sequences of the PCR products (Synergene, Zürich, Switzerland) were identical to *E. cuniculi* sequences deposited in GenBank and identified them as genotype III.

Twenty-one months after these examinations, another adult male Barbary striped grass mouse (animal 4) was found dead in the enclosure. The diagnostic gross post mortem examination revealed a substantially reduced body condition, splenomegaly and enlargement of the mesenteric lymph nodes. The histological examination identified a moderate multifocal granulomatous encephalitis affecting the cortex, and a diffuse granulomatous pneumonia and myocarditis, with pseudocystic protozoan-like structures in endothelial cells, respiratory epithelial cells and macrophages within the lesions. IHC confirmed the protozoa as *E. cuniculi*. Spleen and mesenteric lymph nodes exhibited follicular hyperplasia. Interestingly, neither microsporidia nor inflammatory processes were observed in any other organ.

## Discussion

*E. cuniculi* is an obligate intracellular microsporidian organism that commonly causes subclinical and occasionally lethal infections in rabbits, but can also infect a wide range of hosts, including mice, rats, muskrats, guinea pigs, hamsters, ground shrews, goats, sheep, pigs, horses, domestic dogs, wild and captive foxes, domestic cats, a variety of other carnivores, non-human primates, and humans<sup>2,8,27–31</sup>. *E. cuniculi* infections lead to a variety of different manifestations in different species. In

rabbits, the species which appears to be infected most frequently, neurological, renal, and/or ocular disease are the most common clinical manifestations of encephalitozoonosis. Sudden death is also seen, mainly with fulminant infections, in association with lesions in brain or myocardium<sup>2,32,33</sup>. In Barbary striped mice and other closely related rodents, encephalitozoonosis seems to take a more fulminant, fatal course<sup>34</sup>. A group of Steppe lemmings (*Lagurus lagurus*) presented with weight loss, aggressive behaviour, conjunctivitis and paresis, death occurred within 48 hours. There were no gross changes, but the histological examination revealed a pyogranulomatous inflammatory reaction in brain and kidneys, with intralosomal microsporidian spores. *E. cuniculi* genotype III was identified as the cause<sup>34</sup>. In other, systematically more distant species, such as canids, transplacental infection is more common, therefore, the disease is most frequent in young animals and manifests as anorexia and stunted growth, ataxia, tremors, posterior weakness, and blindness, with progression to circling, aggressive behaviour and convulsions, and in some cases death<sup>10,28,35,36</sup>. In the blue fox and in primates, besides inflammatory processes with intralosomal organisms in brain, kidneys, lungs, liver, and placenta, vasculitis and perivasculitis is a prominent histological feature of *E. cuniculi* infection, indicating its affinity for blood vessels, i.e. vascular endothelial cells<sup>29,30,35–37</sup>.

To our knowledge this is the first case of encephalitozoonosis in Barbary striped mice, confirming the wide host range of *E. cuniculi*. Affected mice showed no changes suggestive of immunosuppression or concomitant disease, they neither exhibited clinical signs prior to death or euthanasia; these findings suggest subclinical infection in the colony, occasionally leading to death in individual immunocompetent animals. In affected mice, infection was associated with a multifocal granu-

*Encephalitozoon cuniculi*  
infection in Barbary  
striped grass mice  
(*Lemniscomys barbarus*)

S. Kitz et al.

lomatous inflammatory response, mainly in brain (3/4 animals), lungs (2/4) and kidneys (1/4), with evidence of an affinity of the microsporidia for endothelial cells in these organs. The granulomatous encephalitis mainly affected cortex and brain stem, indicating a predilection site similar to that in rabbits, and the nephritis was consistent, though less extensive<sup>2,38</sup>. The pathological findings in the brain, such as endothelial cell infection and protozoan uptake by macrophages in association with substantial inflammatory processes, suggest that the brain might be the primary target organ and also the site of persistence of *E. cuniculi* in Barbary striped grass mice. Previous studies have identified the brain as a site where parasites persist for a longer time period than in other organs, e.g. the kidneys<sup>39</sup>. Uptake of protozoa by macrophages/microglia from infected (and destroyed) endothelial cells might hinder elimination of spores and trigger their persistence in the brain<sup>40</sup>. In experimentally infected rats, a decrease or resolution of granulomas in liver, lung or kidneys with time and during disease progression has been described; this was correlated to the dose of infection<sup>31</sup>.

The source of infection in this colony of Barbary striped grass mice was not identified, as the first occurrence of the disease was not associated with any incident, introduction of animals, or changes in housing. The population is closed since it was first established several years back with founder animals from another zoological collection in Germany. Interestingly, the same *E. cuniculi* genotype (type III, “dog strain”) has previously been identified in the same zoo more than 20 years ago, albeit in a colony of emperor tamarins (*Saguinus imperator*)<sup>23</sup>. Any connection between the two infections remains highly speculative, since the mice were not kept at the zoo at this time and are now housed in a building more than 100 m distant from the building of the affected primates. However, wild mice cannot be excluded as a source of the agents as these are often latent, seropositive carrier<sup>1–3</sup>. *E. cuniculi* spores are highly resistant in the environment and can survive several months

under humid conditions, for transmission direct contact with infected individuals is not required, and waterborne infections might be possible, facilitating wide spreading of the parasites in facilities housing or breeding animals<sup>1</sup>.

Due to the clinical and histological presentation of encephalitozoonosis in rodents, the disease is difficult to differentiate from those caused by other protozoan pathogens, such as *T. gondii* and *N. caninum*, of which rodents are natural intermediate hosts and reservoirs<sup>41</sup>. The differential diagnosis in animals that exhibit protozoan-like structures within several organs would include *T. gondii*, *N. caninum*, *Sarcocystis* spp. and *E. cuniculi*. *E. cuniculi* spores and *T. gondii* or *N. caninum* bradyzoites can be differentiated by light microscopy based on their morphology: *T. gondii* and *N. caninum* bradyzoites are 2–4 × 6–8 µm in size, form small cysts (30–100 µm diameter), are Gram-negative, not birefringent and not acid fast; *E. cuniculi* spores are smaller (1.5–2.5 µm) and form large pseudocysts (up to 120 µm diameter), are Gram positive, birefringent and acid fast. Differentiation between *T. gondii* and *N. caninum* is only possible at ultrastructural level, by IHC or based on their genome. However, *T. gondii* antibodies tend to cross react with, for example, *N. caninum*<sup>42</sup> and *E. cuniculi*, whereas antibodies against *E. cuniculi* show no cross-reactivity with *T. gondii*, *N. caninum*, or other protozoa<sup>43</sup>. Therefore, a combined IHC and molecular approach might be required for definite identification of the causative agent<sup>44</sup>.

## Acknowledgements

The authors are grateful to the technical staff of the Histology Laboratory and the Electron Microscopy Unit, Institute for Veterinary Pathology, Vetsuisse Faculty, University of Zurich, for excellent technical support.



## Infection à *Encephalitozoon cuniculi* chez des rats rayés de Barbarie (*Lemniscomys barbarus*)

*Encephalitozoon cuniculi* est un parasite microsporidien intracellulaire obligatoire qui induit généralement des infections subcliniques chez les lapins mais survient également dans toute une gamme d'autres espèces, y compris divers rongeurs, carnivores, humains et oiseaux. Le présent article décrit un cas d'encephalitozoonose chez un groupe de rats rayés de Barbarie (*Lemniscomys barbarus*) dans un zoo. L'étiologie a été confirmée par immunohistochimie et PCR avec séquençage ultérieur. La source de l'infection n'est pas connue.

**Mots-clés:** Encéphalitozoonose, microsporidies, rat rayé de Barbarie (*Lemniscomys barbarus*)

## Infezione da *Encephalitozoon cuniculi* nel topo striato (*Lemniscomys barbarus*)

L'*Encephalitozoon cuniculi* è un parassita intracellulare obbligato che provoca infezioni subcliniche nei conigli, ma che si presenta anche in una serie di altre specie come vari roditori, carnivori, umani e uccelli. Il presente studio descrive l'encefalitozoonosi in un gruppo di topi striati (*Lemniscomys barbarus*) appartenenti alla collezione di un giardino zoologico. L'etiologia è stata confermata da immunoistochimica e PCR con sequenziamento seguente. La fonte dell'infezione è sconosciuta.

**Parole chiave:** Encefalitozoonosi, microsporidi, topo striato (*Lemniscomys barbarus*)

*Encephalitozoon cuniculi* infection in Barbary striped grass mice (*Lemniscomys barbarus*)

S. Kitz et al.

## Literature

- 1 Wasson K, Peper RL. Mammalian microsporidiosis. *Veterinary Pathology*. 2000;37(2):113–128. doi:10.1354/vp.37-2-113
- 2 Harcourt-Brown FM. Encephalitozoon cuniculi infection in rabbits. *Seminars in Avian and Exotic Pet Medicine*. 2004;13(2):86–93. doi:10.1053/j.saep.2004.01.004
- 3 Meredith AL, Cleaveland SC, Brown J, Mahajan A, Shaw DJ. Seroprevalence of Encephalitozoon cuniculi in wild rodents, foxes and domestic cats in three sites in the United Kingdom. *Transboundary and Emerging Diseases*. 2015;62(2):148–156. doi:10.1111/tbed.12091
- 4 Hinney B, Sak B, Joachim A, Kváč M. More than a rabbit's tale – Encephalitozoon spp. in wild mammals and birds. *International Journal for Parasitology: Parasites and Wildlife*. 2016;5(1):76–87. doi:https://doi.org/10.1016/j.ijppaw.2016.01.001
- 5 Didier ES, Vossbrinck CR, Baker MD, Rogers LB, Bertucci DC, Shadduck JA. Identification and characterization of 3 Encephalitozoon cuniculi strains. *Parasitology*. 1995;111(1924):411–421.
- 6 Xiao L, Li L, Visvesvara GS, Moura H, Didier ES, Lal AA. Genotyping Encephalitozoon cuniculi by multilocus analyses of genes with repetitive sequences. *Journal of Clinical Microbiology*. 2001;39(6):2248–2253. doi:10.1128/JCM.39.6.2248-2253.2001
- 7 Talabani H, Sarfati C, Pillebout E, van Gool T, Derouin F, Menotti J. Disseminated infection with a new genotype of Encephalitozoon cuniculi in a renal transplant recipient. *Journal of Clinical Microbiology*. 2010;48(7):2651–2653. doi:10.1128/JCM.02539-09
- 8 Al-Sadi HI, Al-Mahmood SS. Pathology of experimental Encephalitozoon cuniculi infection in immunocompetent and immunosuppressed mice in Iraq. *Pathology Research International*. 2014;2014. doi:10.1155/2014/857036
- 9 Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clinical Microbiology Reviews*. 2005;18(3):423–445. doi:10.1128/CMR.18.3.423-445.2005
- 10 McInnes EF, Stewart CG. The pathology of subclinical infection of Encephalitozoon cuniculi in canine dams producing pups with overt encephalitozoonosis. *Journal of the South African Veterinary Association*. 1991;62(2):51–54.
- 11 Baneux PJR, Pognan F. In utero transmission of Encephalitozoon cuniculi strain type I in rabbits. *Laboratory Animals*. 2003;37(2):132–138. doi:10.1258/00236770360563778
- 12 Weidner E. Interactions between Encephalitozoon cuniculi and macrophages. *Zeitschrift für Parasitenkunde*. 1975;47(1):1–9. doi:10.1007/BF00418060
- 13 Niederkorn JY, Shadduck JA. Role of antibody and complement in the control of Encephalitozoon cuniculi infections by rabbit macrophages. *Infection and Immunity*. 1980;27(3):995–1002.
- 14 Kotkova M, Sak B, Kvetonova D, Kvac M. Latent microsporidiosis caused by Encephalitozoon cuniculi in immunocompetent hosts: A murine model demonstrating the ineffectiveness of the immune system and treatment with albendazole. *PLoS ONE*. 2013;8(4):e60941. doi:10.1371/journal.pone.0060941
- 15 Canning EU, Hollister WS. Microsporidia of mammals – widespread pathogens or opportunistic curiosities? *Parasitology Today*. 1987;3(9):267–273. doi:10.1016/0169-4758(87)90103-7
- 16 Bigliardi E, Sacchi L. Cell biology and invasion of the microsporidia. *Microbes and Infection*. 2001;3(5):373–379. doi:10.1016/S1286-4579(01)01393-4
- 17 Pleshinger J, Weidner E. The microsporidian spore invasion tube. IV. Discharge activation begins with pH-triggered Ca<sup>2+</sup> influx. *The Journal of Cell Biology*. 1985;100(6):1834–1838.
- 18 Weber R, Bryan RT, Schwartz DA, Owen RL. Human microsporidial infections. *Clinical Microbiology Reviews*. 1994;7(4):426–461.
- 19 Didier ES, Weiss LM, Cali A, Marciano-Cabral F. Overview of the presentations on microsporidia and free-living amebae at the 10th international workshops on opportunistic potists. *Eukaryotic Cell*. 2009;8(4):441–445. doi:10.1128/EC.00302-08

*Encephalitozoon cuniculi*  
infection in Barbary  
striped grass mice  
(*Lemniscomys barbarus*)

S. Kitz et al.

- <sup>20</sup> Sundermann CA, Estridge BH, Branton MS, Bridgman CR, Lindsay DS. Immunohistochemical diagnosis of *Toxoplasma gondii*: potential for cross-reactivity with *Neospora caninum*. *The Journal of Parasitology*. 1997;83(3):440. doi:10.2307/3284407
- <sup>21</sup> Sager H, Fischer I, Furrer K, Strasser M, Waldvogel A, Boerlin P, Audigé L, Gottstein B. A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. *Veterinary Parasitology*. 2001;102(1):1–15. doi: 0.1016/S0304-4017(01)00524-6
- <sup>22</sup> Dubey JP, Barr BC, Barta JR, Bjerkås I, Björkman C, Blagburn BL, Bowman DD, Buxton D, Ellis JT, Gottstein B, et al. Redescription of *Neospora caninum* and its differentiation from related coccidia. *International Journal for Parasitology*. 2002;32(8):929–946. doi:10.1016/S0020-7519(02)00094-2
- <sup>23</sup> Guscetti F, Mathis A, Hatt J-M, Deplazes P. Overt fatal and chronic subclinical *Encephalitozoon cuniculi* microsporidiosis in a colony of captive emperor tamarins (*Saguinus imperator*). *Journal of Medical Primatology*. 2003;32(2):111–119. doi:10.1034/j.1600-0684.2003.00016.x
- <sup>24</sup> Brown KL, Gossner A, Mok S, Mabbott NA. The effects of host age on the transport of complement-bound complexes to the spleen and the pathogenesis of intravenous scrapie infection. *Journal of Virology*. 2012;86(1):25–35. doi:10.1128/JVI.05581-11
- <sup>25</sup> Cassaing S, Bessièrès MH, Berry A, Berrebi A, Fabre R, Magnaval JF. Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. *Journal of Clinical Microbiology*. 2006;44(3):720–724. doi:10.1128/JCM.44.3.720-724.2006
- <sup>26</sup> Asakura T, Nakamura S, Ohta M, Une Y, Furuya K. Genetically unique microsporidian *Encephalitozoon cuniculi* strain type III isolated from squirrel monkeys. *Parasitology International*. 2006;55(2):159–162. doi:10.1016/j.parint.2006.02.002
- <sup>27</sup> Patterson-Kane JC, Caplazi P, Rurangirwa F, Tramontin RR, Wolfsdorf K. *Encephalitozoon cuniculi* placentitis and abortion in a Quarterhorse mare. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.* 2003;15(1):57–59. doi:10.1177/104063870301500113
- <sup>28</sup> Snowden KF, Lewis BC, Hoffman J, Mansell J. *Encephalitozoon cuniculi* infections in dogs: a case series. *Journal of the American Animal Hospital Association*. 2009;45(5):225–231. doi:10.5326/0450225
- <sup>29</sup> Zeman DH, Baskin GB. *Encephalitozoonosis* in squirrel monkeys (*Saimiri sciureus*). *Veterinary Pathology*. 1985;22(1):24–31. doi:10.1177/030098588502200104
- <sup>30</sup> Juan-Sallés C, Garner MM, Didier ES, Serrato S, Acevedo LD, Ramos-Vara J a, Nordhausen RW, Bowers LC, Parás a. Disseminated *encephalitozoonosis* in captive, juvenile, cotton-top (*Saguinus oedipus*) and neonatal emperor (*Saguinus imperator*) tamarins in North America. *Veterinary Pathology*. 2006;43(4):438–46. doi:10.1354/vp.43-4-438
- <sup>31</sup> Müller-Dobles UU, Herzog K, Tanner I, Mathis A, Deplazes P. First isolation and characterisation of *Encephalitozoon cuniculi* from a free-ranging rat (*Rattus norvegicus*). *Veterinary Parasitology*. 2002;107(4):279–285. doi:https://doi.org/10.1016/S0304-4017(02)00163-2
- <sup>32</sup> Harcourt-Brown FM, Holloway HKR. *Encephalitozoon cuniculi* in pet rabbits. *The Veterinary Record*. 2003;152(14):427–31.
- <sup>33</sup> Künzel F, Gruber A, Tichy A, Edelhofer R, Nell B, Hassan J, Leschnik M, Thalhammer JG, Joachim A. Clinical symptoms and diagnosis of *encephalitozoonosis* in pet rabbits. *Veterinary Parasitology*. 2008;151(2):115–124. doi: 10.1016/j.vetpar.2007.11.005
- <sup>34</sup> Hofmannová L, Sak B, Jekl V, Mináriková A, Škorič M, Kváč M. Lethal *Encephalitozoon cuniculi* genotype III infection in Steppe lemmings (*Lagurus lagurus*). *Veterinary Parasitology*. 2014;205(1):357–360. doi: 10.1016/j.vetpar.2014.07.008
- <sup>35</sup> Meng X, Zheng J, He X, Jia H, Zhang Y. First Characterization in China of *Encephalitozoon cuniculi* in the blue fox (*Alopex lagopus*). *Journal of Eukaryotic Microbiology*. 2014;61(6):580–585. doi:10.1111/jeu.12135
- <sup>36</sup> Åkerstedt J, Nordstoga K, Mathis A, Smeds E, Deplazes P. Fox *Encephalitozoonosis*: Isolation of the agent from an outbreak in farmed blue foxes (*Alopex lagopus*) in Finland and some hitherto unreported pathologic lesions. *Journal of Veterinary Medicine, Series B*. 2002;49(8):400–405. doi:10.1046/j.1439-0450.2002.00588.x
- <sup>37</sup> Nordstoga K, Westbye K. Polyarteritis nodosa associated with *nosematosis* in blue foxes. *Acta Pathologica Microbiologica Scandinavica Section A Pathology*. 2009;84A(3):291–296. doi:10.1111/j.1699-0463.1976.tb00102.x
- <sup>38</sup> Cox JC, Hamilton RC, Attwood HD. An investigation of the route and progression of *Encephalitozoon cuniculi* infection in adult rabbits. *The Journal of Protozoology*. 1979;26(2):260–5. doi:10.1111/j.1550-7408.1979.tb02776.x
- <sup>39</sup> Leipig M, Matiassek K, Rinder H, Janik D, Emrich D, Baiker K, Hermanns W. Value of histopathology, immunohistochemistry, and real-time polymerase chain reaction in the confirmatory diagnosis of *Encephalitozoon cuniculi* infection in rabbits. *Journal of Veterinary Diagnostic Investigation*. 2013;25(1):16–26. doi:10.1177/1040638712466394
- <sup>40</sup> Cox JC, Gallichio HA. Serological and histological studies on adult rabbits with recent, naturally acquired *encephalitozoonosis*. *Research in Veterinary science*. 1978;24(2):260–261.
- <sup>41</sup> Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*. 2009; 35(3):221–70. doi:10.1080/10408410902989837
- <sup>42</sup> Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. *International Journal for Parasitology: Parasites and Wildlife*. 2015;4(2):216–238. doi:10.1016/j.ijppaw.2015.04.002
- <sup>43</sup> Habenbacher B, Klang A, Fragner K, Dinhopf N, Künzel F, Weissenböck H. Comparative evaluation of specific methods for labeling of *Encephalitozoon cuniculi* in paraffin wax-embedded tissue samples. *Journal of Veterinary Diagnostic Investigation*. 2012;24(2):370–375. doi:10.1177/1040638711435142
- <sup>44</sup> Künzel F, Joachim A. *Encephalitozoonosis* in rabbits. *Parasitology Research*. 2010;106(2):299–309. doi:10.1007/s00436-009-1679-3

## Corresponding author

Anja Kipar  
Institute of Veterinary Pathology, Vetsuisse Faculty  
University of Zurich  
Winterthurerstrasse 268  
CH-8057 Zurich  
+41 (0) 44 635 8587  
E-Mail: anja.kipar@uzh.ch